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1. Your reference: 15.78174

2. Patent application number *(The Patent Office will fill in this part)*: 0215529.9

04 JUL 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*:

Inovio AS
Oslo Research Park
Gaustadalleen 21
N-0349 Oslo
Norway

P01/7700 0.00-0215529.9

Patents ADP number *(if you know it)*:

If the applicant is a corporate body, give country/state of incorporation: Norway

8418717 001

4. Title of the invention: Electroporation Needle Holder

5. Name of your agent *(if you have one)*: Frank B. Dehn & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*:

179 Queen Victoria Street
London
EC4V 4EL

Patents ADP number *(if you know it)*: 166001

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

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Continuation sheets of this form	-
Description	11
Claim(s)	3
Abstract	1
Drawing(s)	5 +5 JML

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Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
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Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature Philippa Power Date 4 July 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Philippa Power
020 7206 0600

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78174.617

Electroporation Needle Holder

The present invention relates to delivery by electroporation, i.e. the process of introducing substances into cells during or after the application of an electric field. More particularly, the present invention relates to a device for use in delivery by electroporation.

Electroporation is used for example in the treatment of cancer or in gene therapy. Electroporation provides a method of delivering pharmaceuticals or nucleic acids (e.g. DNA) into cells, e.g. skeletal muscle cells. Thus for example the muscle may be electrically stimulated at the same time or shortly after the pharmaceutical or DNA is injected. This method works on the principle that cells act as an electrical capacitor generally unable to pass current. Subjecting the cells to an electric field creates transient permeable structures or micropores in the cell membrane. These pores are large enough to allow the pharmaceuticals and/or DNA to gain access to the cells. With time, the pores in the cell membrane close and the cell once again becomes impermeable.

Various devices for effecting electroporation have been suggested. US 6,208,893 discloses an electrode template apparatus having a plurality of bores through which a plurality of needle electrodes extend, each bore being separately connected to a conductor so that each of the electrodes can be connected to a power supply in use. An insulating portion can be provided along the midportion of each electrode so as to isolate the body tissue adjacent the insulated part of the needle from the electric field produced by the electrode in use. Further, one or more of the needle electrodes may be hollow and can include openings through which medicinal substances can be injected into the body tissue.

In the device of US 6,208,893, the needle electrodes are inserted axially from above into the respective bores in use and are removed by being drawn axially outward after use. The present inventors have identified a problem with the use of such a device in which the bores become contaminated with the blood of an animal or person when the needles are withdrawn after use as the tips of the needles pass through the bores. Thus, the apparatus can only be reused after very thorough disinfection which is time consuming and expensive.

The present invention seeks to provide a device which overcomes this problem. In a first aspect, the present invention provides a device for use in electroporation comprising a housing formed in two or more parts, wherein the parts are moveable relative to one another to open and close the housing, and a groove is formed in a surface of at least one of said parts in such a way as to form a bore extending through the housing when the housing is closed. Preferably the bore is adapted to receive a needle in use and the needle can be inserted and removed from the bore by opening the housing.

Thus, as the needle can be removed from the bore by opening the housing and so lifting it out of an open groove, there is no need to remove the needle from the bore by pulling it out in the axial direction. Consequently blood and any other bodily fluids left on the tip of the needle after use need not be brought through the bore and so the housing will not be contaminated as in the prior art devices.

The parts of the housing could for example be held together in the closed position by a removable belt extending around the outside of the housing. Preferably however, the parts are hingedly attached to one another. This has the advantage of making the housing particularly easy to open and close.

The housing could for example be formed in four parts which make up the quarters of a cuboid, each part having a groove with the cross section of a quadrant formed at the inner corner thereof. Alternatively, the housing could be formed in two parts, with a groove having for example a semi-circular or square cross section formed on the inner surface of one part while the surface of the other part is flat. Preferably however the housing is formed of two parts, a groove of semicircular cross section being provided on the inner surface of each part and being positioned to form a bore of circular section from the two grooves when the housing is closed. It will be appreciated that in this arrangement, the parts of the housing can be hingedly attached together at one end thereof in a manner allowing simple manufacture and use of the device. Further, the circular cross section of the bore is particularly advantageous as the needles to be held therein are normally circular in cross section.

Still more preferably, the housing is formed to receive two needles in two respective bores. Although the device could be used with any number of needles, two needles are often required to carry out electroporation and so this is a particularly preferred arrangement.

The needles could be connected to an electric power supply by standard means such as cables attached to an end of the needle extending out of the housing. Preferably however an electrical contact is provided within the or each bore so that a needle within the bore is brought into contact with an electrical power supply when the housing is closed. This has the advantage that a user need not spend time connecting a needle to a power supply by attaching cables etc. and so is much quicker and simpler to use.

Still more preferably, the device is configured so as to lock the needle in position within the bore when the housing is closed in use. Thus, no additional means

need be provided to stop the needle from moving relative to the housing during insertion of the needle into the body tissue to be treated and the subsequent electroporation process.

In one preferred embodiment, a foot pedal could be provided to activate the power supply when required for electroporation. This has the advantage that a user would have their hands free at all times to hold the device and the needle(s) in place in an animal or person being treated. It will be appreciated however that alternative means such as a switch provided on the needle holder could be provided for activating and deactivating the power supply.

The device of the invention could be used with any standard known, approved needles and injection assemblies or syringes.

In one preferred embodiment, the device could be used with one or more needles, wherein each said needle is surrounded by an insulating sheath, the sheath having one or more apertures formed along the length thereof. The use of such insulated needles has the advantage of reducing the production of edge effects when the needle is used as an electrode.

Preferably, the same needle is used for injecting a substance into the body tissue to be treated and applying an electric field. Where necessary however, the needle could be withdrawn from the sheath arranged within a bore of the housing after injection of a substance into the body tissue to be treated and substituted by an electrode rod for carrying out the electroporation. This would be advantageous for example to avoid the release of unwanted metal ions by the needle which could be caused by the provision of an electric charge on the needle. In this embodiment, the electrode rod could be arranged to be completely surrounded by an insulating sheath to avoid the production of edge effects by the electric field in use.

Further, the insulating sheath arranged within the bore would protect the bore from contamination by blood and/or other bodily fluids as the needle was withdrawn axially from within the bore and sheath.

Preferably, even if the needle is not completely withdrawn from the sheath after injection of a substance into the body tissue, the needle is still axially moveable relative to the sheath. This allows the needle to be withdrawn inside the sheath after injection so that it is fully surrounded by the sheath before the application of an electric field. This has the advantage of further reducing the production of edge effects by the electric field in use.

The sheath could be formed of any electrically insulating and biologically compatible material. Preferably however, the sheath is formed from polytetrafluoroethylene (Teflon^{RTM}).

Preferably, the needles used for injection of a substance into the body tissue to be treated are attached to syringe devices via which injection is carried out. It would also be possible however for the needles to be provided separately for attachment to injection means at an appropriate time.

Preferably, the device is provided with means for determining the depth of insertion of a needle into the body tissue to be treated in use and for automatically commencing injection of a substance into the body tissue to be treated when a desired depth of the needle has been reached.

Preferably a moveable contact can be provided on the device such that in use, the contact determines when the needle has been inserted to a sufficient depth into the body tissue to be treated and then causes injection of a substance to commence. This allows automatic injection of a substance to commence when the needle reaches the correct depth in the body tissue to be treated. The injection can be carried out either while

the needle is stationary or while it is continuing to be inserted.

Still more preferably, the moveable contact further determines when the needle has been inserted to the maximum depth at which injection should be carried out and then causes injection of the substance to stop. In this way it is possible for the substance to be automatically and accurately injected over the height of tissue over which an electric field will be produced in use.

Viewed from a further aspect, the present invention provides a method of electroporation treatment of a human or non-human animal (e.g. a mammal, bird or reptile), said method comprising inserting a needle held in a device according to the invention into tissue (e.g. muscle tissue) in said animal, injecting an active agent (e.g. a pharmaceutical or nucleic acid) through the needle into the tissue, applying an electric field between the needle and an electrode, removing the needle from the tissue and opening the housing of the device to remove the needle therefrom.

Preferably, the needle could be pushed further into the tissue after injection and before the application of an electric field to enable the electric field to be applied over the full height of injected fluid.

It will be appreciated that the electrode could be provided by a second needle held in a or the device according to the invention. Alternatively, the electrode could be a different type of electrode which had been inserted into the body tissue or an electrode which had been applied to the skin surface.

It will further be appreciated that the needle could be any known approved form of needle or any other type of needle described herein.

In an alternative preferred method of treatment, the needle is removed from the device according to the invention after injection and replaced by an electrode,

an electric field being applied between the two electrodes before the electrode is removed.

The device according to the invention could for example be used in the method of WO 98/43702, the contents of which are herein incorporated by reference. Preferably, the device would be used in an electroporation method in which a square bipolar electric pulse is applied to the electrode.

Preferred embodiments of the invention will now be described by way of example only and with reference to the accompanying drawings in which:

Figure 1 is a perspective view of a device according to the invention in the open position;

Figure 2 is a perspective view of the device of Figure 1 in the closed position;

Figure 3 is a schematic plan view of a part of a device according to the invention holding a needle and injection device;

Figure 4 is a schematic elevation view of an alternative needle and injection device for use with the device of the invention;

Figures 5a to 5c are schematic side elevational views showing three stages in a method of electrophoretic treatment using a device according to the invention.

As shown in Figure 1, the device comprises a housing made up of two halves 2,4 which are joined together by a hinge 6. Each half 2,4 of the housing is a rectangular solid and the hinge 6 is provided between adjacent end faces thereof so that the upper plane rectangular surfaces of each half of the housing can be pivoted towards each other until the upper surface 8 of the first half 2 lies directly above the upper surface 10 of the second half 4. In this position, the housing is said to be closed and this is shown in Figure 2.

From Figure 1, it can be seen that recesses or grooves are formed in the upper surfaces 8,10 of each of

the two halves 2,4. Each groove is semi-circular in cross section and has a wider portion 12 extending from a first side 14 of the housing half which leads into a narrower portion 16 which extends to the other side 18 of the housing half. Thus, in use the needle 20 of a syringe device fits into the narrower portion 16 while the syringe or injection part 22 adjacent the needle fits into the wider portion 12 as shown in Figure 3.

The upper surface 8 of the first half 2 of the housing has two recesses of the type described above formed therein which are laterally spaced from one another. Two recesses are also formed in the upper surface 10 of the second half 4 at corresponding locations such that, when the housing is closed so that the first 8 and second 10 surfaces are arranged one above the other, the recesses in the first and second surfaces join to form two bores 23 within which respective needles and syringe or injection devices may be held.

Also as shown in Figure 1, an electrical contact element 24 is provided in the narrower part 16 of each recess in the first half 2 of the housing. The electrical contact elements 24 are connected to an electrical power source V and arranged so that a needle placed within the recess will automatically be brought into contact with the electrical contact element when the housing is closed.

The device shown and described with reference to Figures 1 and 2 can be used with any standard approved needle and syringe device such as for example the Sterile EO CE0123, Sterican^{RTM} 0.40x40 mm BL/LB, 27Gx1½" available from Braun.

In an alternative embodiment, the device can be used with syringe devices including needles which are surrounded by insulating sheaths. A syringe device of this type is shown in Figure 4. As can be seen, the device includes a needle 26 and a Teflon^{RTM} sheath 28. As

shown in Figure 4, the insulating sheath 28 which surrounds the needle has three apertures 30 spaced apart from one another in the axial direction and provided along the length of the sheath. A fluid container 32 including a piston 34 is provided at one end of the needle for injecting fluid therethrough. In one embodiment, the needle is axially moveable relative to the sheath so that after it has been inserted into the body tissue to be treated, the needle is withdrawn into the sheath. This avoids the formation of harmful edge effects when an electric field is applied to the needle. Known cannula devices which are already on the market and so have marketing approval can be used to provide the needle and sheath assemblies of the device, the only modification which is required being the formation of the apertures 30 in the sheaths. Thus, the use of such commercially available cannulas can ensure rapid and inexpensive regulatory clearance. One example of a known cannula device which could be used is the 0.8/25mm diameter Venflon^{RTM} sold by BOC Ohmeda AB of Helsingborg, Sweden.

If desired, means may be provided to sense when the needles 26 are at the correct depth in the muscle or body tissue for injection of the DNA to begin and to automatically move the pistons 34 to effect the injection. These means comprise a moveable skin contact 36 which contacts the skin S as shown in Figures 5a to 5c. As the needles 26 are inserted into the muscle or body tissue to be treated, the contact 36 is pushed upwardly towards the housing 14. The contact member 36 is attached to a lever mechanism consisting of a substantially vertical link 38 extending upwardly from the contact member 36 and a lever 40 which is attached at a first end to the vertical link 38. The lever 40 is attached at its other end to means 42 for causing the pistons 34 to move downwardly. The lever is adapted to pivot about a point 44 located between the two ends of

the lever 40. Thus, as the contact 36 moves upwardly relative to the housing 24 in use, the lever 40 pivots causing the piston moving means 42 to push the pistons down gradually so as to effect injection of the fluids over the height of the needles being inserted. As shown, the piston moving means comprise a vertical member 46 attached to the lever 40 to move downwardly as the lever pivots and a cross piece 48 attached to the other end of vertical member 46 to push the piston tops down as it moves downwardly with the vertical member.

The relative location of the skin contact 36 and lever mechanism can be adjusted to ensure injection of the fluids once the needles have reached the muscle tissue and while they are being inserted further into the tissue to ensure a uniform distribution of sample in the area around the electrodes in the muscle.

Figure 5a shows the device before the pistons have been pushed down with the tips of the needles only inserted into the skin. Figure 5b shows the device when the needles are fully inserted to the required depth in the muscle tissue and the pistons 34 have been fully depressed by the action of the lever mechanism. Figure 5c shows the device once the needles have been attached to a power supply V after injection of the fluids. As shown, the syringes have been removed although this is not essential.

In alternative embodiments, lasers or sensors could be used to detect the depth of insertion of the needles and automatically initiate injection of the fluids at a desired depth.

The contact or sensors can be further adapted to sense when the needles 26 have reached a depth in the body tissue at which injection of the fluids should stop so as to ensure that fluid is only injected into the height of body tissue to which an electric field will be applied in use.

A method of electroporation treatment using the

device of Figures 1 and 2 will now be described. This method could be carried out on any human or non-human animal. A required dose of DNA (which could for example be 100 μl) is provided in each fluid container 32. Then the syringe devices are inserted into respective recesses 12,16 in one half 2 of the housing and the housing is closed so that the needles are held firmly in place in the respective bores formed by the recesses. The needles are then inserted into the body tissue as shown at Figure 5a. The needles are pushed down to the correct depth for injection of the DNA and this is then carried out. After the injection, the needles are then pushed slightly further down into the body tissue and the electric power supply V is activated by a foot pedal (not shown) to apply an electric field via the needles.

After the electric field has been applied, the needles are removed from the body tissue and the housing is opened so that the needles can be lifted out of the recesses. The housing is then ready to be reused with new needles.

The embodiments of the electroporation device described above are preferred embodiments only to which various modifications could be made. For example, although the needles have been described as being attached to a syringe arrangement, it will be appreciated that the needles and syringe part could be provided separately. Further, although the housing has been described as being formed in two halves each having two recesses formed therein, it will be appreciated that it could be formed by any number of parts which allowed the needles to be removed from the housing without pulling out in the axial direction. Further, it could be adapted to hold any desired number of needles. Thus, the scope of the invention is not limited by the embodiments of the device as described above but rather is defined by the scope of the appended claims.

Claims

1. A device for use in electroporation, the device comprising a housing formed in two or more or parts, wherein the parts are moveable relative to one another to open and close the housing, and a groove is formed in a surface of at least one of said parts in such a way as to form a bore extending through the housing when the housing is closed.

2. A device for use in electroporation as claimed in claim 1, wherein the bore is adapted to receive a needle in use and the needle can be inserted into and removed from the bore by opening the housing.

3. A device for use in electroporation as claimed in claim 1 or 2, wherein the parts are hingedly attached to one another.

4. A device for use in electroporation as claimed in claim 1, 2 or 3, wherein the housing is formed of two parts, a groove of semicircular cross section being provided on the inner surface of each part.

5. A device for use in electroporation as claimed in any preceding claim, wherein the housing is formed to receive two needles in two respective bores.

6. A device for use in electroporation as claimed in any preceding claim, wherein an electrical contact is provided within the or each bore so that a needle within the bore is brought into contact with an electrical power supply when the housing is closed in use.

7. A device for use in electroporation as claimed in any preceding claim, wherein a foot pedal is provided to activate the power supply in use.

8. A device for use in electroporation as claimed in any preceding claim, wherein one or more needles are provided and each said needle is surrounded by an insulating sheath.

9. A device for use in electroporation as claimed in claim 8, wherein one or more apertures are formed along the length of the sheath.

10. A device as claimed in claim 8 or 9, wherein the needle is axially moveable relative to the sheath.

11. A device as claimed in claim 8, 9 or 10, wherein the needle is adapted to be withdrawn from the sheath arranged within a bore of the housing after injection of the substance into the body tissue to be treated and substituted by an electrode rod for carrying out the electroporation.

12. A device as claimed in any of claims 8 to 11, wherein the sheath is formed from polytetrafluoro-ethylene.

13. A device as claimed in any preceding claim, wherein the device is provided with means for determining the depth of insertion of a needle into the body tissue to be treated in use and for automatically commencing injection of a substance into the body tissue to be treated when a desired depth of the needle has been reached.

14. A device as claimed in claim 13, wherein a moveable contact is provided on the device such that in use, the contact determines when the needle has been inserted to a sufficient depth into the body tissue to be treated and then causes injection of a substance to commence.

15. A device as claimed in claim 14, wherein the moveable contact further determines when the needle has been inserted to the maximum depth at which injection should be carried out and then causes injection of the substance to stop.

16. A method of electroporation treatment of a human or non-human animal, said method comprising inserting a needle held in a device as claimed in any preceding claim into tissue in said animal, injecting an active agent through the needle into the tissue, applying an electric field between the needle and an electrode, removing the needle from the tissue and opening the housing of the device to remove the needle therefrom.

17. A method as claimed in claim 16, wherein the needle is pushed further into the tissue after injection and before the application of an electric field.

18. A method as claimed in claim 16 or 17, wherein the electrode is provided by a second needle held in a or the device as claimed in any of claims 1 to 12.

19. A method of electroporation treatment of a human or non-human animal, said method comprising inserting a needle held in a device as claimed in any of claims 1 to 15 into tissue in said animal, injecting an active agent through the needle into the tissue, removing the needle from the tissue and the device and replacing it with an electrode, applying an electric field between the electrode and a further electrode, removing the electrode from the tissue and opening the housing of the device to remove the electrode therefrom.

ABSTRACT

Electroporation Needle Holder

A device for use in electroporation is provided comprising a housing formed in two or more parts, wherein the parts are hingedly attached to one another to open and close the housing, and a groove is formed in a surface of at least one of said parts in such a way as to form a bore extending through the housing when the housing is closed, wherein the bore is adapted to receive a needle in use and the needle can be inserted and removed from the bore by opening the housing.

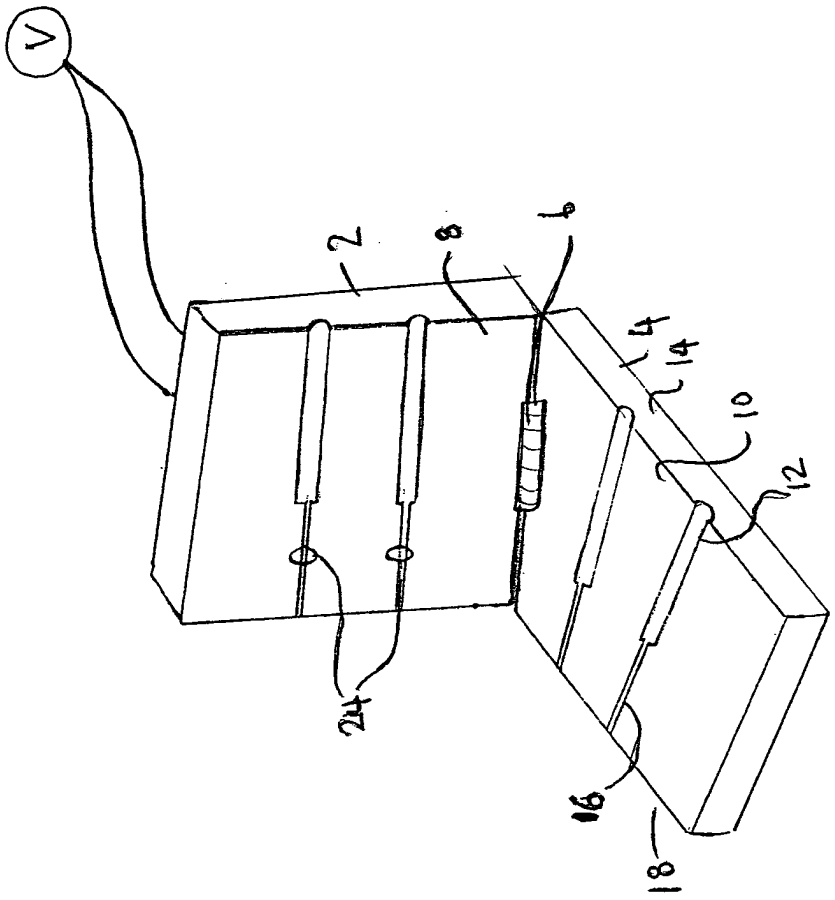


FIG. 1

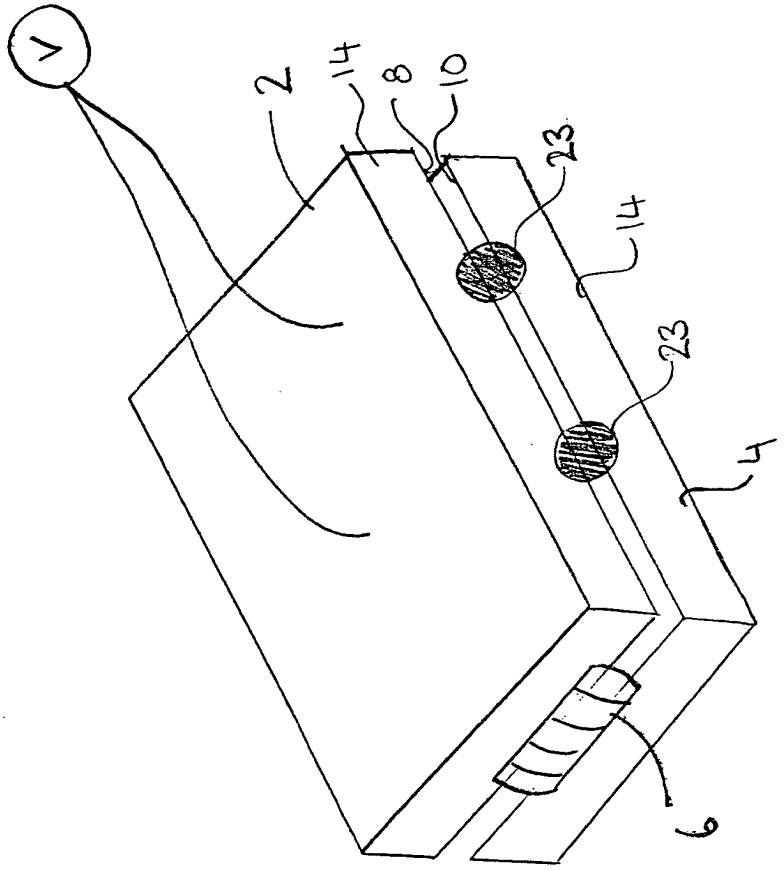


FIG. 2

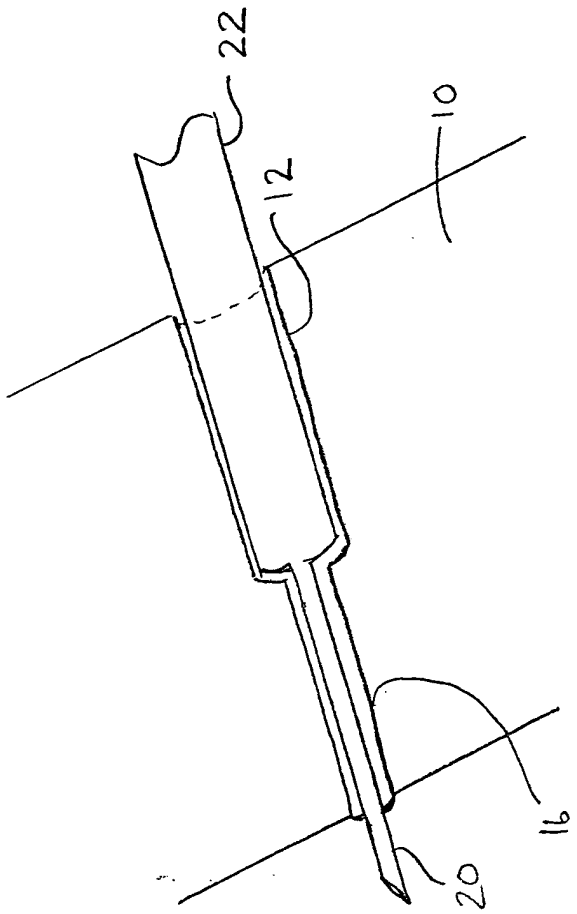


FIG. 3

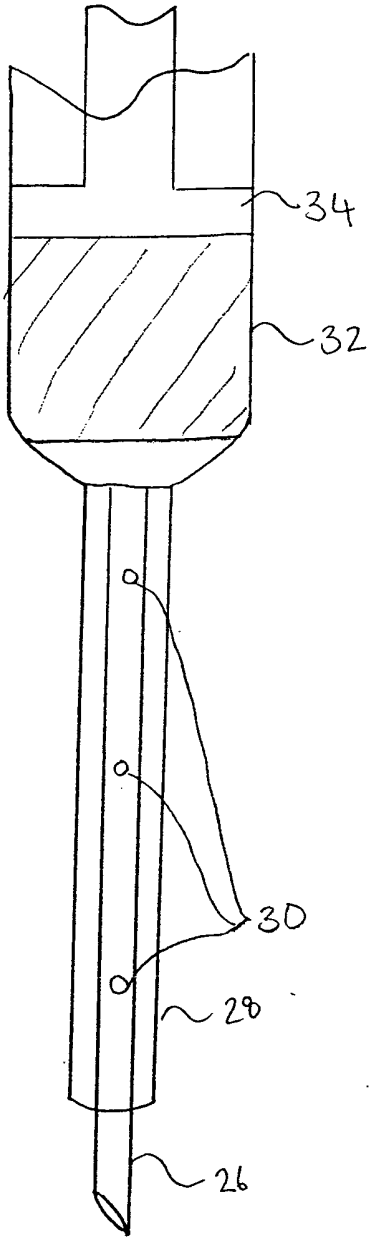


FIG. 4

FIG. 5a

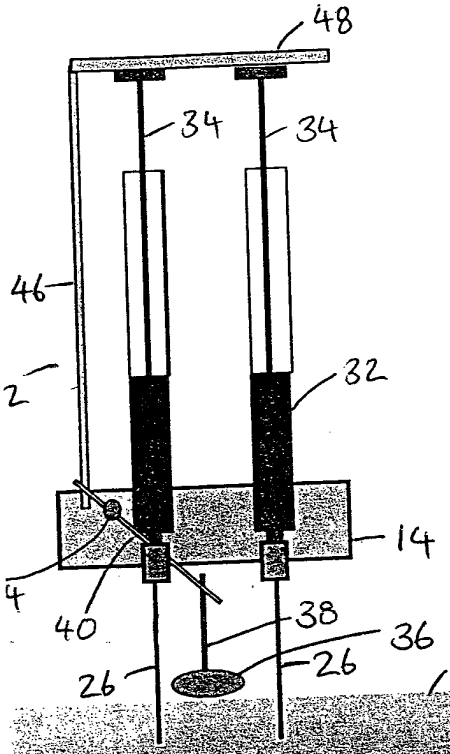


FIG. 5b

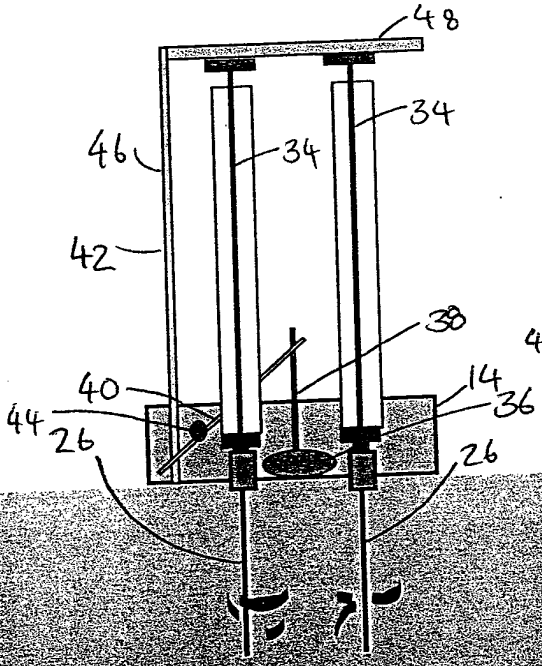
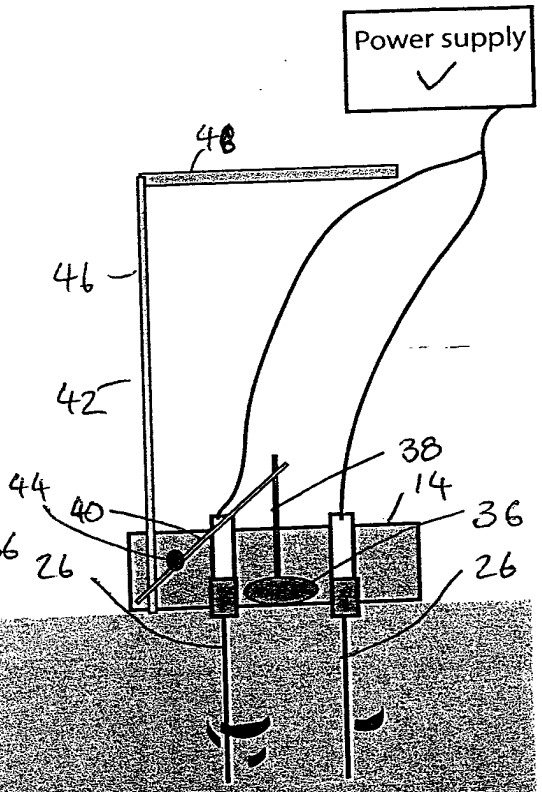


FIG. 5c



Muscle tissue